

REMARKS

Claims 17-26 are pending in the present application.

Favorable consideration of this application, as presently amended, is respectfully requested.

The present invention provides

The rejection of Claims 1-6 and 11-16 under 35 U.S.C. §112, first paragraph ("written description"), is traversed.

The Office has alleged that the specification fails to provide an adequate description of how one can improve thermostability of a protein by comparing amino acid sequences of proteins of different function. As the Examiner concedes, the specification discloses practicing the claimed method with amino acid sequences of proteins of similar function to that of the protein being modified (see paper number 12; page 6, lines 16-17). Accordingly, Applicants have rewritten Claims 1 and 2 as new Claims 17 and 23, respectively, to specifically indicate that said comparing in step (i) is between amino acid sequences of proteins belonging to the *same family* and deriving from two or more species, wherein said proteins evolutionarily correspond to each other in a phylogenetic tree. Moreover, to further complete the description of these claims, Applicants have added testing (step (iv)) and selecting (step (v)) steps to Claims 17 and 23.

Applicants direct the Examiner's attention to MPEP § 2163.02:

An objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *In re Gostelli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989).

Applicants, refer the Examiner pages 5, line 3 to page 6, line 15 and Example 1 of the present application. This section of the application provides that "information of amino acid sequences of proteins corresponding to each other ... ". Moreover, it is apparent for those skilled in the art that proteins derived from two or more species correspond each other when these proteins appear at the similar location in the phylogenetic tree designed for each individual species.

Therefore, the present claims do allow the skilled artisan to recognize what has been invented and what is claimed is adequately described in the specification within the meaning of 35 U.S.C. § 112, first paragraph. Accordingly, withdrawal of this ground of rejection is requested.

The rejection of Claims 1-6 and 11-16 under 35 U.S.C. §112, first paragraph ("enablement"), is traversed.

The Office has taken the position that while the specification is enabled for a method of improving the thermostability of 3-isopropyl malate dehydrogenases wherein amino acid sequences of 3-isopropyl malate dehydrogenases and isocitrate dehydrogenases are compared, the specification does not reasonably provide enablement for a method for improving the thermostability of any protein wherein the amino acid sequences of proteins of different functions are compared (pages 7-9 of paper number 12). Applicants respectfully disagree.

MPEP § 2164.01 states:

"The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation."

Applicants submit that one of skill in the art could obtain a protein having improved thermostability, based on the disclosure provided in the specification, without undue experimentation, especially when the disclosure is augmented with the information known in the art.

Further, MPEP § 2164.04 states:

“A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.”

At pages 4-13, the Applicants fully describe the present invention in generic terms so as to enable the artisan to practice the inventive method. Moreover, at pages 13-29, Applicants provide painstaking detail of the inventive method along with a practical example teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented.

The Examiner appears to call into question the objective truth of the broad-spectrum utility of the present invention. In particular, the Examiner appears disturbed by the supposedly limited number of examples drawn to distinct proteins. In order to demonstrate that the method of the present invention works with proteins other than those described in Examples of the present application and that those skilled in the art could practice the claimed invention based on the description of the present specification, Applicants submit the following (see “Summary of the Invention” at pages 2-3):

- The method of the present invention does not depend on the particular properties of proteins currently existing.

- The method of the present invention is based on the concept that the ancestors common to eubacteria, eukaryotes and archaebacteria might be ultra-thermophilic bacteria, after reviewing the fact that many organisms that properly grow at a temperature of 80°C or above are located at the root of a phylogenetic tree by 16S rRNA.
- The inventors have completed the present invention on the basis of an idea that for designing and producing a thermostable protein, it is more important that the amino acid sequence of ancestral protein is estimated and mimicked than that only the sequence and the higher-order structure of protein of a thermophilic bacterium are analyzed and mimicked.

Further evidence of the enablement of the present invention is provided in the executed Declaration under 37 C.F.R. §1.132 submitted herewith. In this Declaration, Applicants demonstrate that the thermostability of a glycyl tRNA was improved by practicing the claimed method. In paragraphs 1-2, Applicants provide purification of genomic DNA and subsequent purification of the glycyl tRNA synthetase (GlyRS). As described in paragraph 3, the production of the phylogenetic tree and estimation of the ancestral type amino acid residues was obtained by alignment of GlyRS obtained in paragraph 2 with amino acid sequences of $\alpha 2$ type GlyRSs from a variety of organisms obtained from DNA databank using the ClustalX program. Specifically, the neighbor-joining method was employed to establish the phylogenetic tree for the $\alpha 2$ type GlyRSs (step (i) of the present claims) from which the ancestral amino acids were identified (step (ii) of the present claims).

Next, the amino acid residues identified in the previous step were compared to the amino acid sequence of one of the GlyRS proteins and 15 positions were selected as residues to examine the ancestral reversion (step (iii) of the present claims). Upon creation of

ancestral-type mutant GlyRS proteins (paragraph 4), these proteins were expressed and purified (paragraph 5). The pure ancestral-type mutant GlyRS proteins were then measured for their respective thermostabilities (paragraph 5; step (iv) of the present claims). As demonstrated in Table 1 (see page 12 of the Declaration under 37 C.F.R. §1.132) and summarized on page 11, of the 7 ancestral-type mutant GlyRS proteins tested, the thermostability of two were unchanged compared to the wild-type enzyme, one exhibited a decreased thermostability, and four had an increased thermostability. This latter four ancestral-type mutant GlyRS proteins complete the inventive method of selecting a protein having improved thermostability (step (v) of the present claims).

Based on the foregoing, Applicants submit that the present claims are fully enabled by the specification and the common ability available in the art and as such withdrawal of this ground of rejection is requested.

The rejection of Claims 1-6 under 35 U.S.C. §102(b) over Lehmann et al is traversed. Claims 1-16 have been canceled and rewritten as new Claims 17-26.

Lehmann et al disclose increasing the thermostability of a fungal phytase by constructing a consensus enzyme based on 13 fungal phytase sequences (see Abstract and page 51, column 2). Applicants submit that this disclosure by Lehmann et al is fundamentally different from the present invention and, therefore, cannot affect the patentability of the present invention.

At the outset, Applicants note that “designing an ancestral protein” (present invention) differs from “designing a consensus protein” (Lehmann et al). Specifically, designing an ancestral protein takes the shape of phylogenetic tree into account while designing a consensus protein does not. It is conceivable that the consensus sequence and ancestral sequence may overlap, but only if the consensus sequence is conserved even at the root area

in the phylogenetic tree. Moreover, a sequence may be “the ancestral sequence” for the given protein, yet not be the “consensus sequence.” In order to demonstrate this proposition, Applicants provide the following Example (Table A, below) showing that “an ancestral sequence” is different from “a consensus sequence.” The ancestral sequence in Table A is determined using the method described in the present specification at page 8, line 4 to page 9, line 7, Example 1 in the specification and Figure 3.

Table A: Consensus sequence and ancestral sequence for *Thermus thermophilus* IPMDH and ICDH

Proteins and Species	Amino Acid Sequence	
	70	131
IPMDH Aquifex aeolicus	GAVG	IRENT
IPMDH Arabidopsis thaliana	CAIG	VRELT
IPMDH Escherichia coli K12	GSVG	VRELT
IPMDH Thermotoga maritime	GSVG	VRELS
IPMDH Bacillus subtilis	GAVG	VRELT
IPMDH Saccharomyces cerevisiae	GAIG	VRELV
IPMDH Thermus thermophilus	GSVG	VRELT
IPMDH-Pyrococcus abyssi ²	GAIG	VRENT
IPMDH Sulfolobus tokodaii	GPVG	VRENT
Consensus	GAVG	VRELT
Ancestral	GPVG	VRENT

Proteins and Species	Amino Acid Sequence	
	70	131
ICDH Saccharomyces cerevisiae ¹	GLWH	IRENT
ICDH Arabidopsis thaliana	GGLK	IRENT
ICDH Saccharomyces cerevisiae ²	GPLA	IRENT
ICDH Thermus thermophilus	GPLE	VRENV
ICDH Caldococcus noboribetus	GPLT	FRENT
ICDH Sulfolobus tokodaii	GPLE	FRENT
ICDH Thermus aquaticus	GPLT	FRENT
ICDH Escherichia coli K12	GPLT	FRENS
ICDH Bacillus subtilis	GPLT	FRENT
Consensus	GPLT	FRENT
Ancestral	GPVG	VRENT

Residue No. 71 of IPMDH is "A" for the consensus sequence while the residue is "P" for the ancestral sequence. Similarly, residue No. 134 of IPMDH is "L" for the consensus sequence while the residue is "N" for the ancestral sequence. The T_m value of A71P mutant and L134N mutant was 91.7°C and 91.8°C, respectively, while the wild type has T_m value of 88.8°C. This indicates the thermostability was increased by 2.9°C or 3.0°C, respectively. For this case, it should be noted that the consensus sequence is identical to the wild type sequence, which means the ancestral type mutant protein was more improved in thermostability than the consensus type (wild type) protein. Thus, the effect of "constructing an ancestral sequence" is strictly different from the effect of "constructing an consensus sequence."

Moreover, based on Table A above, it should be noted that if the consensus sequence should be determined by considering both of IPMDH and ICDH, which have the similar function as the examiner pointed out, the consensus sequence may vary depending on which sequences of IPMDH and ICDH should be taken into account most significantly. This indicates that designing a consensus sequence is likely to be affected by the genes used to establish this consensus sequence, whereas designing an ancestral sequence is less prone to this bias.

Additionally, it should be noticed that the present invention does not intend to modify a protein such that it functions at *high* temperature, but the present invention seeks to *improve* the thermostability of a protein. Specifically, an object of the present invention is to produce a protein functioning at a *higher* temperature than the naturally occurring wild type protein. Accordingly, the Examiner's opinion that "not all proteins are intended to be functional at high temperature" (see paper number 12, page 9, lines 1-3) is of no moment.

In summary, Lehmann et al only discloses constructing a *consensus* phytase and does not disclose or suggest constructing an *ancestral* sequence. The standard for determining anticipation requires that the reference “must teach every element of the claim” (MPEP §2131). Therefore, the absence of any disclosure or suggestion by Lehmann et al of constructing an ancestral sequence as presently claimed would necessarily make this reference fail to anticipate the present invention. Moreover, Applicants submit that the present invention would not even be obvious in view of Lehmann et al as this reference fails to even realize the difference between “designing an ancestral protein” and “designing a consensus protein,” much less the advantages flowing therefrom.

Applicants respectfully request withdrawal of this ground of rejection.

The rejection of Claims 1-6 and 11-16 under 35 U.S.C. §112, second paragraph, is obviated by appropriate amendment. Claims 1-16 have been canceled and rewritten as new Claims 17-26.

With respect to the Examiner’s rejection based on the recitation of the phrase “species which evolutionarily correspond to each other in a phylogenetic tree,” Applicants have amended this phrase to recite “wherein said proteins evolutionarily correspond to each other in a phylogenetic tree.” Applicants submit that in so doing the meaning of the relationship of “evolutionarily correspond” is now clear.

The Examiner rejected original Claims 1 and 3 for omitting essential steps. The Examiner suggested adding the testing and selecting steps (old Claims 2 and 4) into Claims 1 and 2. Applicants wish to thank Examiner Ramirez for this helpful suggestion, which they have adapted in new Claims 17 and 23 presented herein above. As stated above with respect to the rejection under 35 U.S.C. §112, first paragraph, Applicants have further amended original Claims 1 and 2 (now Claims 17 and 23, respectively) to introduce the limitation of

the range of proteins that should be compared. Namely, the proteins that should be compared are limited to the proteins belonging the same family (limitation of old Claims 5 and 6).

Finally, regarding “the protein” appearing in old Claims 11 and 12 (new Claims 19 and 20), Applicants have now clearly indicated that this term refers to “the protein being tested for improved thermostability.”

Based on the foregoing, Applicants submit that the pending claims are definite within the context of 35 U.S.C. §112, second paragraph. Accordingly, Applicants request withdrawal of this ground of rejection.

The objections to the specification and to Claim 3 are obviated by the present amendment.

Withdrawal of this ground of objection is requested.

Applicants submit that the present application is now in condition for allowance.
Early notification of such action is earnestly solicited.

Respectfully submitted,

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